translocation. For that, the structural order of lipid membranes was investigated by measuring fluorescence polarization of membrane-bound fluorophores such as 1,6-phenyl-1,3,5-hexatriene (DPH) and 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene p-toluene sulfonate (TMA-DPH) in the presence and absence of different cephalosporin generations as a function of temperature. Location studies have been also carried out using electron paramagnetic resonance (EPR) spectroscopy, valuable tool for collecting information on the dynamics of lipids and membrane structure. The results obtained suggest that the incorporation of these antibiotics into DMPC and DMPG bilayer does not significantly modify their transition temperature whereas perceptible changes in the cooperativity of the phospholipid transition are observed.

782-Pos Board B661

Inversion of Lipid Bilayer Surface Charge by Trivalent Cations: Probing with Single-channel Conductance and Kinetics

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The conductance of gramicidin A is sensitive to the charge of the lipid bilayer in which it resides. We used this property to probe the effects of lanthanum^{3+}, hexaamminecobalt^{3+}, and spermidine^{3+} on the surface charge of phosphatidylserine (PS) bilayers. Addition of trivalent cations to negatively-charged PS bilayers reduced gramicidin conductance below the conductance seen for neutral phosphatidylcholine bilayers, to a level nearly as low as for positively charged trimethylammonium propane bilayers. This suggests that trivalent cations can overcompensate the negative surface charge of the PS bilayer. Complementary zeta-potential measurements of PS liposomes with trivalent cations also suggested charge inversion. There were differences in the concentrations required to invert charge among the different cations, with lanthanum^{3+} the most potent and spermidine^{3+} the least potent. We also find that the rate of channel formation is sensitive to the surface concentration of permeating ions. Our interpretation is that gramicidin monomers in a bilayer exist in different configurations and that the equilibrium between these configurations depends on the cation binding within monomers. The difference in occupancy of monomers by cations makes channel formation dependent on the surface potential.

783-Pos Board B662

Breakdown of Charged Lipid Asymmetry as a Result of Lipidic Pore Formation

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Negatively charged lipids are usually located in the inner leaflet of the plasma membrane. Their appearance in the outer leaflet is known to correlate with several physiological and pathological conditions in cells. Understanding how membrane lipids lose their asymmetric transmembrane distribution and achieve their nonrandom distribution in cells is a key challenge in cell biology. Negatively charged lipids do not spontaneously exchange between the two leaflets of a lipid bilayer because the polar headgroups cannot readily cross the hydrophobic membrane interior. We hypothesized that the formation of a transient hydrophilic lipidic pore in the membrane leads to diffusive translocation of negatively charged lipids through the pore to the opposite membrane leaflet. To test this hypothesis, we established a variation of the inner field compensation technique for time resolved measurements of membrane boundary potentials in asymmetric bilayer lipid membranes formed by the Montal-Mueller method. External application of electric fields across the bilayer induced transient conductive states. We observed fast transitions between these different conductance levels, reflecting opening and closing of meta-stable lipidic pores. Comparison of the capacitance minimization potential for different asymmetric membranes before and after pore formation confirmed negatively charged lipids transfer across the bilayer. We also constructed a model governing lipid flow rate based on pore analysis and lipid lateral diffusion rate. Together, our study provides a new tool to monitor loss of membrane asymmetry and our results indicate that lipid transfer can occur through lipidic pores.

784-Pos Board B663

Percolation Thresholds for Diffusing Particles of Nonzero Radius: Circular Obstacles in the Two-dimensional Continuum

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Lateral diffusion in the plasma membrane is obstructed by proteins bound to the cytoskeleton. The most important parameter describing diffusion in the presence of immobile obstacles is the percolation threshold, where long-range connected paths disappear and the long-range diffusion coefficient D goes to zero. To describe obstructed diffusion, it is more accurate to find the threshold directly than to extrapolate a low-density expansion in the obstacle concentration to find the concentration at which $D = 0$. The thresholds are well-known for point diffusing particles on various lattices or the continuum. But for particles of nonzero radius, the threshold depends on the excluded area, not just the obstacle concentration. Earlier results [Saxton, Biophys J 64 (1993) 1053] for the triangular lattice showed a very rapid decrease in the threshold as the radius of the diffusing particle increases, but a lattice model gives very low resolution. The current work finds the percolation threshold for circular obstacles in the two-dimensional continuum as a function of the radius of the diffusing species. The thresholds are obtained by a Monte Carlo method based on the Voronoi diagram for the obstacles. Each Voronoi bond is by definition the path equidistant from the nearest pair of obstacles, so the separation of that pair determines whether a diffusing particle of a given diameter can traverse that bond. For point obstacles, then, one can choose a threshold corresponding to the diameter of the diffusing particle, set the conductivity of all bonds narrower than that diameter to zero and all wider bonds to one, and test for bond percolation on the resulting Voronoi diagram. The results are used to find the thresholds for lipids and for proteins of different diameters. (Supported by NIH grant GM038133)

785-Pos Board B664

Effects Of Hydration On The Dynamics Of Water In Lipid Bilayer Systems: A Molecular Dynamics Study

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The properties of interlamellar water are critically important to the structure and function of biological membranes. Recent developments in femtosecond infrared spectroscopy on membrane systems at various hydration levels have opened the possibility of direct comparison between experiment and molecular dynamics simulations on the same time scales. The interpretation of experimental findings can benefit from a detailed computational study of water solvation structure and dynamics of inter-lamellar water at these hydration levels. In this presentation, we report molecular dynamics simulations of 1-palmitoyl-2-oleoyl-phosphatidylcholine POPC bilayers in the liquid-crystalline state and at three hydration levels. Simulations were performed in the canonical ensemble using the GROMACS software package. The extent to which water is influenced by the presence of membrane depends on the hydration level. We found the anisotropic diffusion constant of lipid water exhibits interesting crossover behavior as the water molecule moves from the head group region toward the bulk region. The anisotropic hydrodynamic diffusion of water is explained by structural perturbation of the water hydrogen bond network by the lipid. Radial distribution function, spatial distribution function, and power spectra of water are calculated to consolidate our interpretation. This work was partially supported by the National Science Foundation under Grant No. DGE-0221680.

786-Pos Board B665

Photophysical Properties of Novel Ruthenium Metal-Ligand Complexes incorporated in Lipid Membrane Bilayers

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We have designed and synthesized novel metal-ligand complexes with amine- or acyl- reactive functional groups. These complexes have potential as luminescent probes to investigate bio-macromolecular dynamics on the submicrosecond-to-microsecond timescale. This time scale is of interest, for example, for analysis of the motions associated with large macromolecular assemblies and interactions involving membrane-bound proteins. Here we report the photophysics and structural properties of (1) the complex $[HRu(CO)(dicarboxy\text{-}bipyridyl)(PPh_3)_2]^+$ $[PF_6]^-$ conjugated to the lipid dipalmitoyl-phosphatidylethanolamine (DPPE) and (2) the complex $[(CF_3CO_2)Ru(CO)\text{-}(5aminophen)(Ph_2PC_2H_2PPh_2)]^+$ $[PF_6]^-$ conjugated to cholesterol. The conjugated complexes were incorporated in unilamellar lipid membrane vesicles to investigate the photophysical properties of these probes in the membrane environment and to evaluate the utility of these probes for investigating the physical properties of lipid membranes.

787-Pos Board B666

Evaluating Gramicidin A Channel Backbone Dynamics by Molecular Dynamics and Nuclear Magnetic Resonance

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